

Use of Ion Exchange Resins in Soil and Plant Testing for Micronutrient Availability. R.S. Tejowulan, J.J. Schoenau, and J.R. Bettany, Department of Soil Science, University of Saskatchewan.

## INTRODUCTION

Much effort has been directed towards the development of soil and plant tissue tests, to determine the concentration of available micronutrients in soil and plant materials, quickly and accurately (Sims and Johnson, 1991; Jones, 1991; Martens and Lindsay, 1990). Extracting solutions containing a chelate or weak acid are commonly used to assess micronutrient availability in soils and plants. Realization that such methods often remove larger amounts of the available nutrient pool than do the plants, has led to concern about their accuracy as indices of plant availability.

Ion exchange resins may be a more suitable approach to extracting ions from soils and plants than conventional extracting solutions. Earlier research has reported that ion exchange resin in a loose bead form can successfully extract the fraction of exchangeable Cu, Zn, and Mn that is taken up by plants during the growing period. However, the loose bead form suffers from separation problems and therefore is not suitable for routine analysis. With the presence of exchange resin in a membrane form however, extraction techniques may be potentially easier in routine analysis, and also more efficient because of the large surface area available for adsorption and exchange.

The objectives of this research were : 1) To develop a new technique to extract bioavailable micronutrients from soil and plant tissue materials using ion exchange membrane, 2) To validate the developed techniques, and 3) To provide indexes of critical micronutrient levels in soils and plant tissue for the particular methods, crop(s), and micronutrients.

## MATERIALS AND METHODS

### *Measurement of Micronutrient Availability in Soils* Selection of soils

Thirty two Saskatchewan and fourteen Indonesian soil samples were selected for soil test experiment. The selected soils varied in texture and had a wide range of micronutrient availability including both deficient and sufficient levels.

### Ion exchange resin membranes

The ion exchange membranes used in this study were anion exchange resin in a membrane form (AEM), B.D.H product no. 55164. To fit the requirements for soil extraction and elution, membrane sheets were uniformly cut from their original packaged size 12 x 12 cm to form rectangular strip sizes of 3 cm x 6 cm. In a preliminary experiment, this 36 cm<sup>2</sup>-strip surface was found to extract measurable amounts of micronutrient ions and conveniently fit inside the 125 and 50 ml centrifuge tubes used for the extraction and elution.

### Membrane pretreatment

Strips of anion exchange membrane were saturated with 0.01 M-DTPA-disodium salts or 0.01 M-EDTA-disodium salts, to form a chelating cation exchange membrane referred to as AEM-DTPA or AEM-EDTA, respectively. Results from preliminary experiments suggested that the AEM-DTPA and AEM-EDTA were very effective in removing metal ions and other polyvalent ions from soils. This saturation procedure was conducted by placing approximately 200 of the AEM strips in 700 ml of the saturating solution overnight, or by shaking in the solution for about 45 minutes.

#### Soil extractions

The laboratory developed membranes (AEM-DTPA and AEM-EDTA) were then tested for their ability to extract micronutrients from soil suspension and by direct burial in soils. Conventional methods e.g. DTPA-TEA (Lindsay and Norvel, 1978) and EDTA-(NH<sub>4</sub>)CO<sub>3</sub> (Trierweiler and Lindsay, 1969) were included for comparison purposes.

#### Growth chamber trial

Canola (*Brassica napus* L. cv. Westar) was grown in the 32 Saskatchewan and 14 Indonesian soils. Canola was chosen for this experiment because it is sensitive to Cu, Zn, and Mn deficiencies in soil (Karamanos et al., 1986). Four treatments were established for the experiment: minus Cu, minus Zn, minus Mn, and minus Fe. Each pot was watered daily with deionized water such that the soil was always between 70 to 100% field capacity. The growth chamber temperatures were maintained at  $25 \pm 1^\circ\text{C}$  during the 16 h day-period and  $18 \pm 1^\circ\text{C}$  during the 8 h night-period for the duration of the experiment. The plants were harvested 52 days after seeding.

#### Measurement of Micronutrients in Plant Tissue

##### Sample size and amounts

Leaf samples (*Vicia faba*) were taken for tissue testing at seventh leaf stage (27 days after seeding). Samples of plant tissue of approximately 10 g were taken from the second to fourth leaves of each plant using plastic scissors. The 10 g fresh samples were then cut uniformly into smaller pieces of approximately 5 mm x 5 mm using the scissors.

##### Leaf suspension extraction and elution

The AEM-DTPA and AEM-EDTA membranes were also tested for their ability to extract micronutrient cations from fresh plant tissue suspension. Instead of using a single 3 cm x 6 cm size of membrane as used in the soil testing experiment, the 36 cm<sup>2</sup> membrane was cut into three strips of 1 cm x 6 cm. Elution of the membranes was performed with 1 M-HCl. Leaf water extraction (without membranes) was included as a control.

##### Micronutrient response experiment

Soils were sampled from three sites in Saskatchewan where micronutrient deficiencies were identified from the results of soil tests. A completely randomized block design was established for experimentation and each treatment was replicated 3 times. Fababean (*var. Tick*) and oats (*var. Calibre*) were chosen for their sensitivity to Zn & Fe and Cu & Mn, respectively. Treated pots received additions of Cu, Zn, Mn, and Fe fertilizers at seven rates according to treatments. Growth chamber temperatures, day and night photoperiods, and light intensity were kept at the same conditions as the previous experiment. The oats and fababean were harvested at 85 and 95 days after seeding, respectively.

##### Total Digest

At each harvest, the plants were cut 1 cm above the soil surface and washed with deionized water. Each sample was dried at 60°C for 48 hours in a forced air oven and the total plant dry matter weight was determined. Straw and seed samples were ground to < 425 µm in a Cyclone mill with a nickel impeller in preparation for chemical analysis. Total Cu, Zn, Mn, and Fe were determined in nitric-sulfuric acid-peroxide digests using AAS (Thomas et al., 1967).

##### Statistical Analyses

Linear correlation was employed to examine the relationships between soil test levels and canola uptake, dry matter yield, and conventional test methods. Statistical analysis of data from the plant tissue test experiment mainly involved correlation analyses

of the relationship between plant tissue test values and fababean and oats nutrient uptake as well as between the rates of fertilizer applications and total dry matter yield. Analysis of variance (ANOVA) was used to determine differences among fertilizer rates for fababean (Zn and Fe) and oats (Cu and Mn). The critical tissue levels were determined by constructing a hand-fitted curve on the sample points, and were associated with the breaking point of the curve, or the midpoint of the transition zone.

## RESULTS AND DISCUSSION

### Soil Extraction

#### *Extraction techniques adopted*

##### Soil suspension extraction

Extraction of ions in soil suspension was performed using the AEM-DTPA and AEM-EDTA membrane strips. The procedure involved the extraction of ions from 5 g soil (< 2 mm) using 36 cm<sup>2</sup> of the membrane in 100 ml deionized water for 1 hour, at 20°C. The mixture of soil, membrane, and water was placed in a 125 ml centrifuge tube. The extraction was then carried out using a side by side shaker, at 120 strokes per minute (s.p.m.) shaking speed.

##### Membrane burial

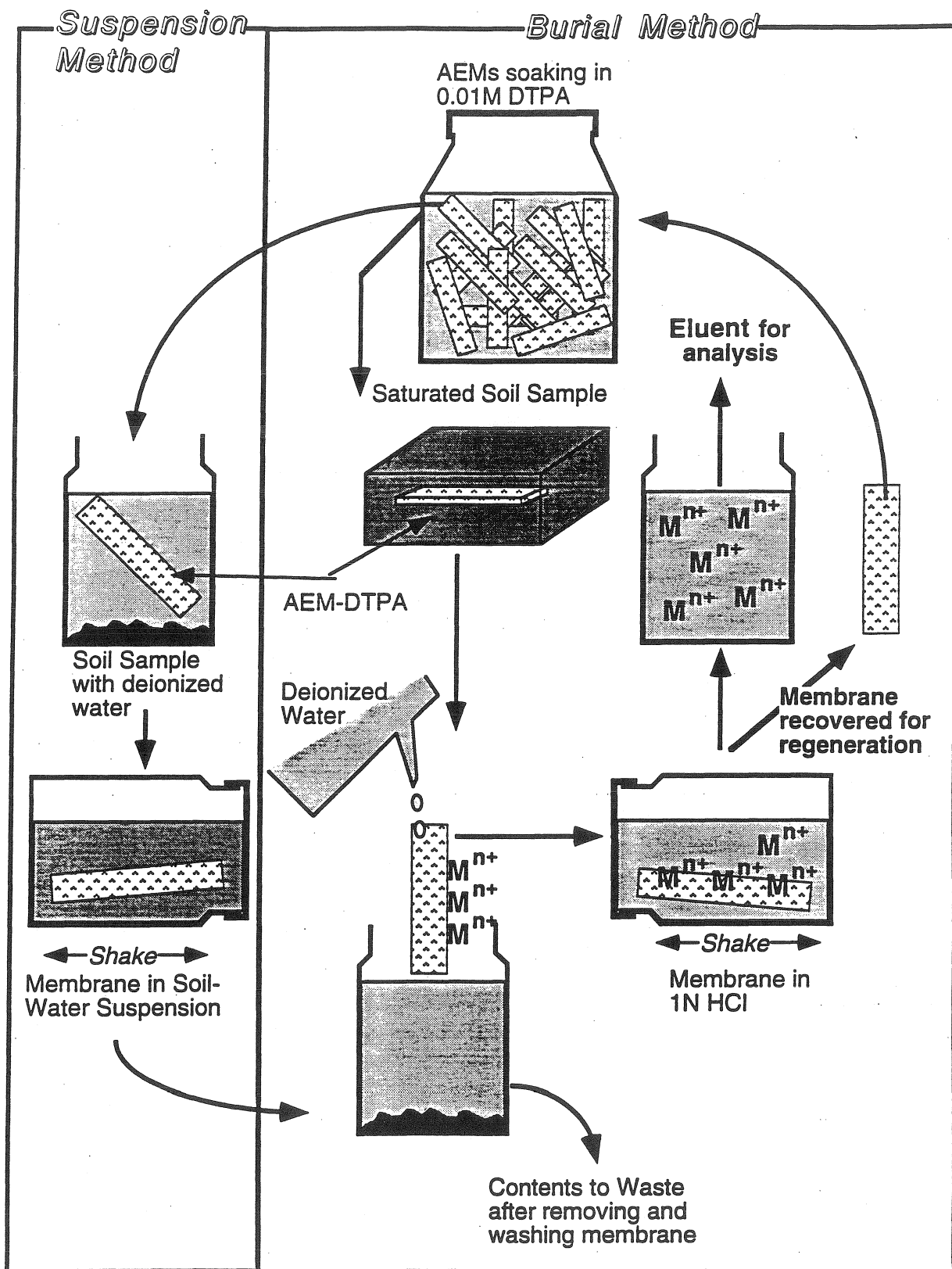
The membrane burial extraction was carried out by burying 36 cm<sup>2</sup> of the membrane (AEM-DTPA or AEM-EDTA) in soil (< 2.0 mm) for 5 minutes. Water was added gradually to the dry soil to ensure that the soil was completely saturated. This step may take about 10 to 15 minutes depending on the soil texture and may allow an equilibrium between soil solution and solid phases of soil to take place. Prior to insertion of the membrane, the soil sample was stirred thoroughly using a stirring rod for about 5 seconds to ensure a homogeneous condition. The membrane was placed horizontally in the soil sample to provide a sink for ions moving upward by force of evaporation, as well as ions moving through the soil solution to the strip surface by diffusion.

##### Elution and regeneration procedures

Following the extraction, membranes were removed from the soil mixture using plastic tweezers and rinsed free of soil with deionized water. The washed membranes were then transferred into a 50 ml centrifuge tube containing 20 ml of 1 M-HCl. The elution (removal of the ions adsorbed to the membrane) was performed using a side by side shaker (120 s.p.m) for 30 minutes. The eluent was transferred into a vial and elements in the eluent were determined by Atomic Absorption Spectrophotometry (AAS) using a Perkin Elmer model 3100. The membranes were removed from the tubes, washed with deionized water, and placed into 0.01 M-DTPA-disodium salts or 0.01 M-EDTA-disodium salts for regeneration. The suspension extraction and membrane burial procedures are illustrated in Figure 1.

##### *Pattern and nature of micronutrient removal*

The micronutrient metal pools extracted by resin may resemble the conventional DTPA or EDTA extractable pools, depending on the kinds of chelating agents used to saturate the membrane. When the membrane is saturated with DTPA, micronutrients should come primarily from exchange sites, dissolved minerals, and soluble organic chelates (Adams and Kissel, 1989; Lindsay and Norvell, 1978). The greater extraction of Cu, Zn, and Mn by AEM-EDTA, relative to AEM-DTPA, can be attributed to the presence of EDTA which will enhance the removal of organically complexed and oxide-bound metals (Adams and Kissel, 1989; Al-Showk et al., 1987). Increases in Cu, Zn, Mn, and



**Figure 1.** Flow Diagram of Soil Suspension Extraction and Burial Procedures using AEM-DTPA membranes

Fe extracted by resin with increased length of shaking time reflects the low solubility of micronutrients. The gradual increase with time suggests that only highly soluble micronutrient forms such as  $\text{CuCO}_3$ ,  $\text{Cu}(\text{OH})_2$ ,  $\text{Cu}_2(\text{OH})_2\text{CO}_3$ ,  $\text{Zn}(\text{OH})_2$  (amorp),  $\text{Mn}(\text{OH})_2$ ,  $\text{Mn}_2\text{O}_3$ ,  $\text{Fe}(\text{OH})_3$  (amorp), and some organic micronutrient complexes are the main micronutrients forms removed in short extraction times. Over longer extraction, amounts extracted may include the less soluble forms such as  $\text{CuFe}_2\text{O}_4$ ,  $\text{Zn}_2\text{SiO}_4$ ,  $\text{MnO}$ , and  $\text{Fe}(\text{OH})_3$ , whereas the relatively stable forms such as  $\text{CuO}$ ,  $\text{ZnFe}_2\text{O}_4$ ,  $\text{MnOOH}$ ,  $\text{MnCO}_3$ ,  $\text{MnO}_2$ ,  $\text{Fe}_2\text{O}_3$ , and  $\text{FeOOH}$  may be released only in equilibrium with depleted soil solution after prolonged shaking or burial periods.

The resin strip method developed has good precision. The average coefficient of variation (C.V.) for three replicate analyses of a 2 mm soil was  $\pm 3.2\%$  for the resin burial method and  $\pm 3.7\%$  for the resin suspension method. Sources of variability include error in subsampling the bulk soil sample as well as in the extraction procedure and ion concentration analysis.

#### *DTPA-TEA and $\text{EDTA}-(\text{NH}_4)_2\text{CO}_3$ Extractions*

The DTPA-TEA soil test was considered a reliable method for estimating the nutritional status of Cu, Zn, Mn, and Fe in alkaline and calcareous soils (O'Connor, 1988; Leggett and Argyle, 1983; Lindsay and Norvel, 1969). Furthermore, the same soil test has also proved useful for estimating metal availability in acid soils (Silviera and Sommers, 1977). Therefore, the test was used in the present study to evaluate the capacity and reliability of the newly developed resin methods in assessing the availability status of soil Cu, Zn, Mn, and Fe as related to predicting micronutrient plant uptake.

The reproducibility of DTPA-TEA method was good with average C.V. values less than 2%. The DTPA-TEA values for Cu, Zn, Mn, and Fe are more reproducible than those obtained with resin methods (C.V. values of about 3.5%).

The  $\text{EDTA}-(\text{NH}_4)_2\text{CO}_3$  soil test has been used successfully to identify soils low in available Cu and Zn (Burridge and Hewitt, 1987; Brown et al., 1971). Particularly with Cu, the test often gives a better estimation of status of the nutrient in soil than the DTPA extractant. In the present experiment, the  $\text{EDTA}-(\text{NH}_4)_2\text{CO}_3$  test produced highly reproducible measurements. The average C.V. of the  $\text{EDTA}-(\text{NH}_4)_2\text{CO}_3$  soil test (2.3%) fell between those of the DTPA-TEA and resin tests.

#### *Comparison Among The Soil Test Methods*

All the methods extracted amounts of Cu, Zn, Mn, and Fe that can be reliably measured using conventional atomic absorption measurement techniques. Except for Mn in Indonesian soils which appeared somewhat low (40%), the EDTA test extracted larger amounts of Cu (190%), Zn (185%), and Mn (106%) than did the DTPA-TEA (Table 1). However, the EDTA test removed less Fe (65%) than the DTPA-TEA.

The burial method extracted lower quantities of micronutrients than did the other methods. Soil Cu, Zn, Mn, and Fe determined by the 5 min. burial varied from a low of 1.0, 0.1, 15, and 8  $\mu\text{g}\cdot 100\text{ cm}^{-2}$  of strip surface area to a high of 6.8, 1.7, 93, and 38  $\mu\text{g}\cdot 100\text{ cm}^{-2}$ , for Cu, Zn, Mn, and Fe, respectively. The 30 min. burial method extracted more micronutrient cations than the 5 min.: approximately 2 times higher for Cu and Zn, 4 times higher for Mn, and 3 times higher for Fe. Direct comparison between burial test values and other methods is not possible because the levels determined by burial are expressed per unit surface area of strip surface rather than per unit weight of soil.

#### *Relationships among the soil test methods*

The amounts of Cu, Zn, Mn, and Fe removed by resin suspension and burial methods were shown to be significantly correlated with the micronutrients removed by the DTPA-TEA or  $\text{EDTA}-(\text{NH}_4)_2\text{CO}_3$  soil tests (Table 2). The AEM-DTPA soil test was most closely correlated to Zn, Mn, and Fe DTPA-TEA values ( $r = 0.89^{***}$ ,  $0.80^{***}$ , and

0.71\*\*\*, respectively). For Cu however, the burial 5 min. was the most closely correlated to the DTPA-TEA ( $r = 0.92^{***}$ ).

Table 1. Means and observed ranges of soil micronutrients extracted by various tests.

Extractants	Mean	Range	Percentage of DTPA value (%)	Mean	Range	Percentage of DTPA value (%)
	SASKATCHEWAN			INDONESIAN		
Cu-DTPA-TEA <sup>†</sup>	0.91	0.21 - 2.36		0.41	0.11 - 0.77	
Cu-EDTA-(NH <sub>4</sub> ) <sub>2</sub> CO <sub>3</sub> <sup>†</sup>	1.41	0.33 - 4.07	156	0.92	0.24 - 1.58	225
Cu-AEM-DTPA <sup>†</sup>	0.62	0.11 - 2.38	67	1.31	0.12 - 2.62	325
Cu-AEM-EDTA <sup>†</sup>	0.90	0.31 - 2.17	100	2.26	0.40 - 3.96	550
Cu-Burial 5 min. <sup>††</sup>	1.94	1.02 - 4.71		3.81	1.71 - 6.80	
Cu-Burial 30 min. <sup>††</sup>	3.91	1.80 - 7.25		7.51	3.51 - 16	
Zn-DTPA-TEA <sup>†</sup>	0.94	0.21 - 2.55		0.51	0.22 - 1.10	
Zn-EDTA-(NH <sub>4</sub> ) <sub>2</sub> CO <sub>3</sub> <sup>†</sup>	1.71	0.57 - 5.33	188	0.92	0.42 - 1.64	180
Zn-AEM-DTPA <sup>†</sup>	0.10	0.05 - 0.20	11	0.22	0.04 - 0.41	40
Zn-AEM-EDTA <sup>†</sup>	0.22	0.11 - 0.35	24	0.33	0.06 - 0.84	66
Zn-Burial 5 min. <sup>††</sup>	0.62	0.13 - 1.72		0.43	0.12 - 1.22	
Zn-Burial 30 min. <sup>††</sup>	1.0	0.51 - 2.02		1.02	0.41 - 3.33	
Mn-DTPA-TEA <sup>†</sup>	11.6	6.2 - 21		21.3	2.02 - 86	
Mn-EDTA-(NH <sub>4</sub> ) <sub>2</sub> CO <sub>3</sub> <sup>†</sup>	20.0	11.8 - 31	172	8.6	1.01 - 20	40
Mn-AEM-DTPA <sup>†</sup>	4.21	1.4 - 7.8	36	3.4	0.21 - 7.3	16
Mn-AEM-EDTA <sup>†</sup>	10.1	2.8 - 21	87	16.2	0.72 - 50	76
Mn-Burial 5 min. <sup>††</sup>	21	15 - 42		27	10 - 93	
Mn-Burial 30 min. <sup>††</sup>	87	58 - 189		93	30 - 312	
Fe-DTPA-TEA <sup>†</sup>	39.4	12.0 - 77		47.1	4.61 - 113	
Fe-EDTA-(NH <sub>4</sub> ) <sub>2</sub> CO <sub>3</sub> <sup>†</sup>	25.9	8.0 - 50	66	29.2	2.91 - 62.7	62
Fe-AEM-DTPA <sup>†</sup>	1.61	0.91 - 2.2	5	2.8	0.81 - 6.3	6
Fe-AEM-EDTA <sup>†</sup>	1.24	0.81 - 2.0	4	3.4	0.51 - 7.9	8
Fe-Burial 5 min. <sup>††</sup>	15	8.1 - 29		22	6.1 - 38	
Fe-Burial 30 min. <sup>††</sup>	40	20 - 86		52	11 - 100	

<sup>†</sup> =  $\mu\text{g}\cdot\text{g}^{-1}$  and <sup>††</sup> =  $\mu\text{g}\cdot 100\text{ cm}^{-2}$  strip surface area

#### *Ability of various methods to predict relative micronutrient availability*

Moderate correlations were observed between Cu uptake by canola and relative predicted availability by soil tests. The  $r$  values of correlations between Cu uptake by canola and relative availability predicted by the Cu-EDTA-(NH<sub>4</sub>)<sub>2</sub>CO<sub>3</sub> soil test were 0.77\*\*\* for Saskatchewan and 0.83\*\*\* for Indonesian soils. With the DTPA-TEA, the  $r$  values were 0.86\*\*\* for Saskatchewan and 0.71\*\* for the Indonesian soil. Among the five procedures, the resin burial 5 min. soil test was the best Cu extractant correlated with Cu canola uptake in Saskatchewan soils ( $r = 0.91^{***}$ ). With Indonesian soils, the AEM-DTPA extractable Cu had the highest  $r$  values (0.83\*\*\*), whereas the DTPA-TEA soil test gave the lowest  $r$  value (0.71\*\*).

Table 2. Regression equations and r values for relationships between micronutrients extracted by resin methods and that of the conventional methods for Saskatchewan and Indonesian soils.

Regression Equation			r
<b>SASKATCHEWAN</b>			
Cu AEM-EDTA	=	0.48825 + 0.28978 Cu EDTA	0.70***
Cu AEM-DTPA	=	- 0.087119 + 0.55866 Cu DTPA	0.76***
Cu BURIAL 5 min.	=	0.0086905 + 0.011454 Cu DTPA	0.92***
Cu BURIAL 30 min.	=	0.0026652 + 0.014526 Cu DTPA	0.78***
Zn AEM-EDTA	=	0.14283 + 0.043605 Zn EDTA	0.74***
Zn AEM-DTPA	=	0.054347 + 0.051586 Zn DTPA	0.89***
Zn BURIAL 5 min.	=	0.0054302 + 0.0031538 Zn DTPA	0.65***
Zn BURIAL 30 min.	=	0.0060646 + 0.004856 Zn DTPA	0.83***
Mn AEM-EDTA	=	- 3.6473 + 0.68934 Mn EDTA	0.59***
Mn AEM-DTPA	=	4.17724 + 0.34735 Mn DTPA	0.80***
Mn BURIAL 5 min.	=	0.10958 + 0.0084497 Mn DTPA	0.52**
Mn BURIAL 30 min.	=	0.40825 + 0.040112 Mn DTPA	0.50**
Fe AEM-EDTA	=	0.73226 + 0.018638 Fe EDTA	0.62***
Fe AEM-DTPA	=	0.93266 + 0.017968 Fe DTPA	0.71***
Fe BURIAL 5 min.	=	0.07678 + 0.0017984 Fe DTPA	0.65***
Fe BURIAL 30 min.	=	0.21455 + 0.0047251 Fe DTPA	0.61***
<b>INDONESIAN</b>			
Cu AEM-EDTA	=	0.83427 + 1.5567 Cu EDTA	0.72**
Cu AEM-DTPA	=	0.38425 + 2.5142 Cu DTPA	0.79***
Cu BURIAL 5 min.	=	0.019427 + 0.049135 Cu DTPA	0.75**
Cu BURIAL 30 min.	=	0.035935 + 0.10443 Cu DTPA	0.72**
Zn AEM-EDTA	=	- 0.03441 + 0.41854 Zn EDTA	0.66**
Zn AEM-DTPA	=	0.0031088 + 0.3265 Zn DTPA	0.76***
Zn BURIAL 5 min.	=	0.00096317 + 0.0056641 Zn DTPA	0.54*
Zn BURIAL 30 min.	=	0.0034353 + 0.013408 Zn DTPA	0.53*
Mn AEM-EDTA	=	0.55305 + 1.8071 Mn EDTA	0.75**
Mn AEM-DTPA	=	2.1045 + 0.060072 Mn DTPA	0.74**
Mn BURIAL 5 min.	=	0.035555 + 0.011072 Mn DTPA	0.94***
Mn BURIAL 30 min.	=	0.17613 + 0.035351 Mn DTPA	0.94***
Fe AEM-EDTA	=	1.5425 + 0.062395 Fe EDTA	0.56*
Fe AEM-DTPA	=	1.3139 + 0.031814 Fe DTPA	0.73**
Fe BURIAL 5 min.	=	0.091725 + 0.0027333 Fe DTPA	0.94***
Fe BURIAL 30 min.	=	0.13071 + 0.0082157 Fe DTPA	0.93***

\*, \*\*, \*\*\* Significant at 0.05, 0.01, and 0.001 probability levels, respectively.



In general, significant positive relationships were found between extractable Zn and canola uptake. All the procedures tested yielded results which were moderately to highly correlated with Zn plant uptake ( $r = 0.64^{**}$  to  $0.88^{***}$ ). The DTPA-TEA and EDTA- $(\text{NH}_4)_2\text{CO}_3$  methods gave a similar  $r$  value ( $r = 0.88^{***}$ ). Relative Zn uptake by canola as predicted by resin tests gave  $r$  values ranging from  $0.69^{***}$  to  $0.80^{***}$  for Saskatchewan and  $0.70^{**}$  to  $0.86^{***}$  for Indonesian soils. The  $r$  values were highest with DTPA-TEA, EDTA- $(\text{NH}_4)_2\text{CO}_3$ , and the AEM-DTPA for Saskatchewan soils ( $r \geq 0.80^{***}$ ) and with the resin burial 5 min. for Indonesian soils ( $r = 0.86^{***}$ ).

All methods appeared capable of differentiating the relative Mn and Fe availability in the selected soils indicated by moderate to high correlation coefficients found between extractable soil Mn ( $0.65^{***}$  to  $0.85^{***}$ ) and Fe ( $0.58^*$  to  $0.84^{***}$ ) with the Mn and Fe canola uptake. A significant relationship obtained with Fe is rather surprising as methods for estimating Fe availability in soils have rarely given a significant relationship with plant uptake and yields. Significant correlations between amounts of Fe extracted from soils by DTPA and amounts taken up by plants were first reported by Brown and Holmes (1956). Since then few studies have reported significant correlations between soil tests and plant Fe uptake. The canola sensitivity to Fe deficiency coupled with the digest method used in this study, in part, may have contributed to the significant results. In contrast, extractable Mn has been used successfully in many studies, particularly for soybean and small grains, to predict crop yield responses to Mn fertilization (Sims, 1986; Sheppard and Bates, 1982; Shuman et al., 1980; Randall et al., 1976). The ability to establish significant relationships between soil Mn and Fe and plant Mn and Fe, respectively, may be attributed to deficiency in the selected soils.

#### *Relationships between Cu, Zn, Mn, and Fe availability indices and plant parameters*

In general, significantly higher amounts of dry matter production and micronutrient uptake were observed in plants grown on soils in which high availability of micronutrient was indicated, whereas lower amounts were produced by the canola crop on soils with low micronutrient availability as predicted by the soil tests.

### **Plant tissue testing**

#### *Development and Evaluation of Resin and Water as Extractants*

##### Leaf suspension extraction and elution

This involved extraction of inorganic and soluble organic ion forms in leaves using AEM-DTPA and AEM-EDTA membrane and water extractants. Approximately 5 g of fresh plant tissue plus 3 membranes (total surface area of  $36 \text{ cm}^2$ ) was added to a 125 mL centrifuge tube along with 50 mL of deionized water. The mixture of the tissue sample, water, and membrane was then shaken on a side by side shaker (220 r.p.m) at two different shaking times, 30 minutes and 1 hour. Following the extraction, the residual leaf tissue was dried for 48 hours at  $60^\circ\text{C}$  and weighed. The membranes were removed from the tube and rinsed with deionized water to separate the tissue sample from the membrane. The washed membranes were then put into a 4 dram vial containing 10 mL of 1 M-HCl and shaken for 30 minutes using a side by side shaker (120 - 220 r.p.m). The membranes were removed from the eluent for regeneration, as described in the previous section. The eluent was measured for Cu, Zn, Mn, and Fe using AAS.

##### Leaf Water Extraction

The extraction of plant tissue with water alone was carried out in a manner similar to that of the plant tissue membrane extraction, except that no membranes were used in the shaking. Instead, following the extraction, the mixtures of plant tissue and water were filtered through a filter paper (Whatmann No. 42) and free micronutrient cations in the extracts were measured using AAS.



#### *Pattern and nature of micronutrient removal*

Results of the analysis indicate that the resin tests developed for plant tissue were highly reproducible. The highest mean coefficient of variation (C.V.) for three replicate samples was less than 10%, with average C.V. less than 4%. Such low C.V.'s may be attributed to (i) the uniformity and reproducibility of the membrane extraction as was shown in the soil experiment and (ii) the uniformity of the tissue samples used. Compared to the resin membrane, the water extraction procedure was not as reproducible with an average C.V. of 12%. High C.V.'s may be attributed to contamination of the filtrate supernatant with organic compounds from the plant tissue which dissolve in the water during the extraction and pass through the filter paper. This was observed, especially with oats, and prevailed even after repeated filtration.

The water extraction may include micronutrients from soluble complexes in the plant tissue such as enzymes and other smaller molecules in addition to the free inorganic ionic states. Such complexes may not be adsorbed to the resin strip. As such, the resin pools may resemble the water extractant but are not likely to be identical in the constituents measured because resin should mainly absorb charged ions in the water-plant mixture released from plant materials. The resin tissue test extracted approximately 25% of the total tissue Cu, 7% of Zn, 20% of Mn, and 8% of total tissue Fe. The water extractant extracted a larger portion of total element in plant tissue than the resin extractant, accounting for 35% of Cu, 12% of Zn, 25% of Mn, and 12% of Fe.

The water procedure extracted micronutrient amounts approximately two times greater than the resin methods. This may be explained by incomplete removal of all the soluble micronutrients by the resin strip. As well, the water extractions may contain some other soluble constituents such as complexes which are not adsorbed by the resin within the given extraction periods (30 min. and 60 min.). Higher extractable values of Cu, Zn, Mn, and Fe from plant tissue observed with AEM-DTPA relative to AEM-EDTA may be related to enhanced removal of soluble organic chelates by DTPA.

#### *Tissue nutrient levels vs uptake values*

In all cases, changes in micronutrient availability induced by fertilizer addition influenced the uptake of micronutrient by the crops. Increasing Cu, Mn, and Fe supplies increased the element uptake up to maximum, where after further increases in the supply decreased uptake. Zinc uptake, however, continually increased with increasing Zn supply.

With AEM-DTPA, AEM-EDTA, and water extractions, the micronutrient extractable from plant tissue was closely related to the micronutrient fertilizer rates applied. In particular, the Zn concentrations extracted by the three procedures increased with increasing rate of Zn fertilizer applied, while for Cu, Mn, and Fe, uptake increased only in lower fertilizer rates. Analysis of total micronutrient content of the whole tops revealed that the highest total uptake of Cu, Zn, Mn, and Fe always occurred in plants with the highest concentration given by the tissue tests (Figure 2). For Zn, the results revealed luxury uptake by fababean, in that increases in Zn plant concentration occurred beyond the point of maximum yield. With Cu, Mn, and Fe, fertilization increased uptake only when the element status in soil was inadequate to support the normal growth, whereas excessive Cu, Mn, and Fe added as fertilizers reduced uptake.

Total micronutrient uptake by plants versus the various tissue levels for Cu, Zn, Mn, and Fe in fertilized soils is plotted in Figure 2. Second order gave better  $r$  values than the simple linear analysis.

The correlation coefficients in general were higher with the resin tissue tests (0.63 to 0.93) than the water procedure (0.69 to 0.83). The AEM-DTPA was superior over the AEM-EDTA in predictions of Cu, Zn, and Mn uptake by plants. Among the four elements, the prediction of plant Zn uptake was the best of the three different methods.

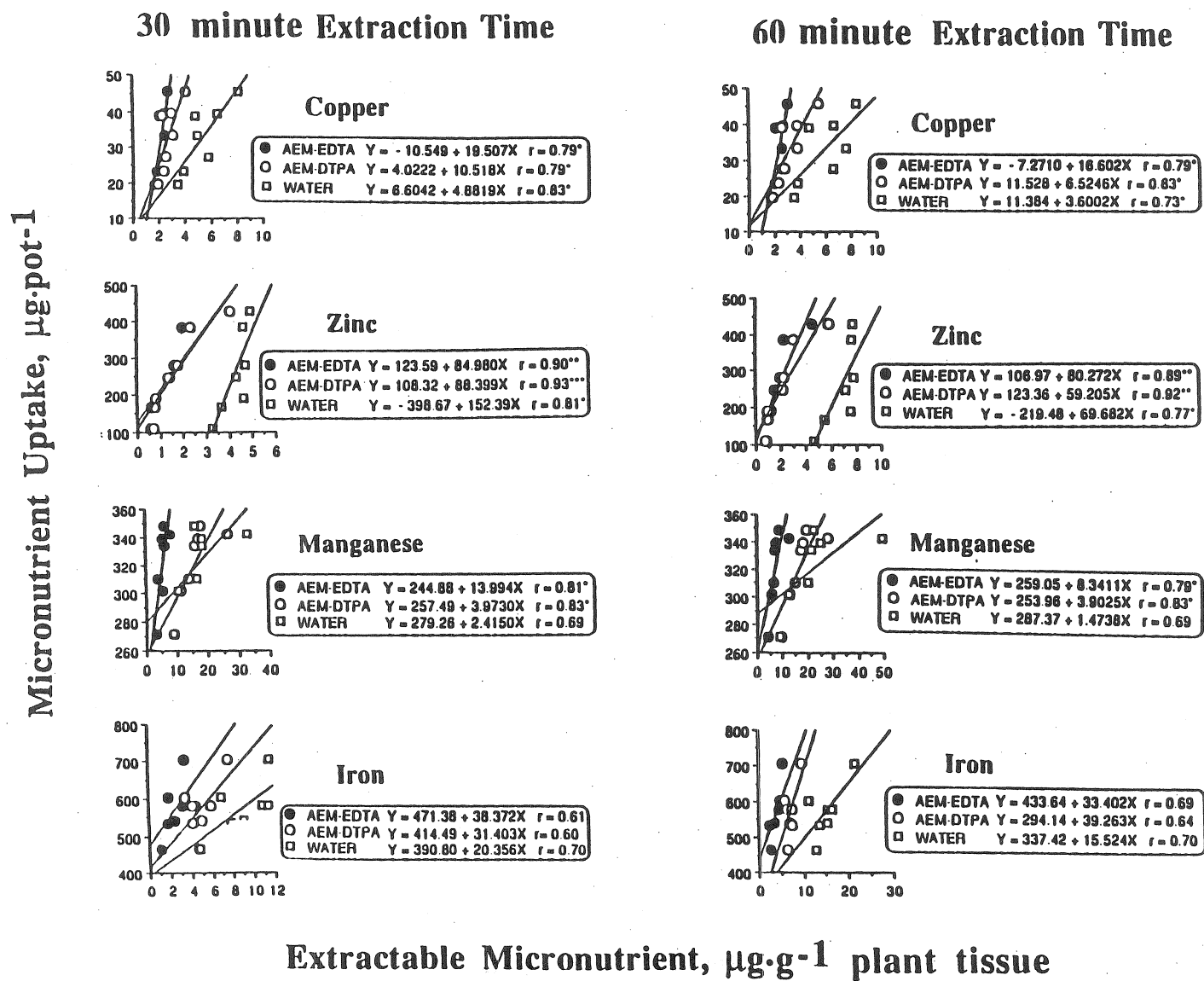


Figure 2. Relationships between micronutrient plant uptake and Cu, Zn, Mn, and Fe extracted by various methods at two different extraction times from fertilized tissue plants.

### Critical test levels

Critical micronutrient concentrations are usually defined as the concentration associated with 90% of maximum yield (Dang et al., 1990). The critical micronutrient levels estimated by the resin membrane and the conventional methods are arranged in Table 3.

Table 3. Critical micronutrient levels for canola as estimated by soil tests.

Soil Test	Micronutrient							
	Cu		Zn		Mn		Fe	
	S	I	S	I	S	I	S	I
DTPA-TEA <sup>†</sup>	0.60	0.30	0.50	0.30	8.11	10	13	15
EDTA-(NH <sub>4</sub> ) <sub>2</sub> CO <sub>3</sub> <sup>†</sup>	0.90	0.70	1.40	0.60	16	15	15	10
AEM-DTPA <sup>†</sup>	0.60	0.60	0.07	0.10	2.12	3.11	0.90	1.01
AEM-EDTA <sup>†</sup>	0.70	1.20	0.20	0.25	5.00	15	1.01	1.02
BURIAL 5 min. <sup>††</sup>	2.02	3.01	0.60	0.30	18	20	13	13
BURIAL 30 min. <sup>††</sup>	4.07	6.02	1.01	0.60	80	60	30	30

<sup>†</sup> =  $\mu\text{g}\cdot\text{g}^{-1}$ ; <sup>††</sup> =  $\mu\text{g}\cdot 100\text{ cm}^{-2}$  strip surface; S = Saskatchewan; and I = Indonesian.

Table 4 shows the estimates of critical micronutrient level as predicted by the various plant tissue tests.

Table 4. Estimates of critical micronutrient level as predicted by various plant tissue tests.

Method	Micronutrient			
	Cu <sup>†</sup>	Zn <sup>††</sup>	Mn <sup>†</sup>	Fe <sup>††</sup>
	----- μg·g <sup>-1</sup> dry matter -----			
30 min. Extraction				
AEM-DTPA*	3.5	2.5	16.0	3.5
AEM-EDTA*	2.4	2.0	6.0	3.5
WATER	5.3	4.6	19.0	7.0
60 min. Extraction				
AEM-DTPA*	3.8	3.1	18.0	5.5
AEM-EDTA*	2.7	2.2	6.4	5.0
WATER	6.6	6.2	27.0	10.5
Total Tissue Concentration	11	31	65	85

\* Extraction by a 36 cm<sup>2</sup> surface resin

<sup>†</sup>, <sup>††</sup> oat and fababean, respectively.

## CONCLUSIONS

Anion exchange membrane pretreated with chelating agent is a promising method for assessing micronutrient availability, both in soil and tissue testing. The developed methods are rapid, simple and well correlated with plant uptake. The resin procedures have high potential for simultaneous determination of several elements, micro and macro. In soil experiments, all methods were significantly related to plant uptake for Cu, Zn, Mn, and Fe. The AEM-chelate 5 minute burial method developed is simple and rapid, and offers advantages over the conventional methods. In plant tissue testing experiments, the membrane methods developed were significantly related to plant uptake for Zn, Cu, and Mn, particularly strong for Zn.

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